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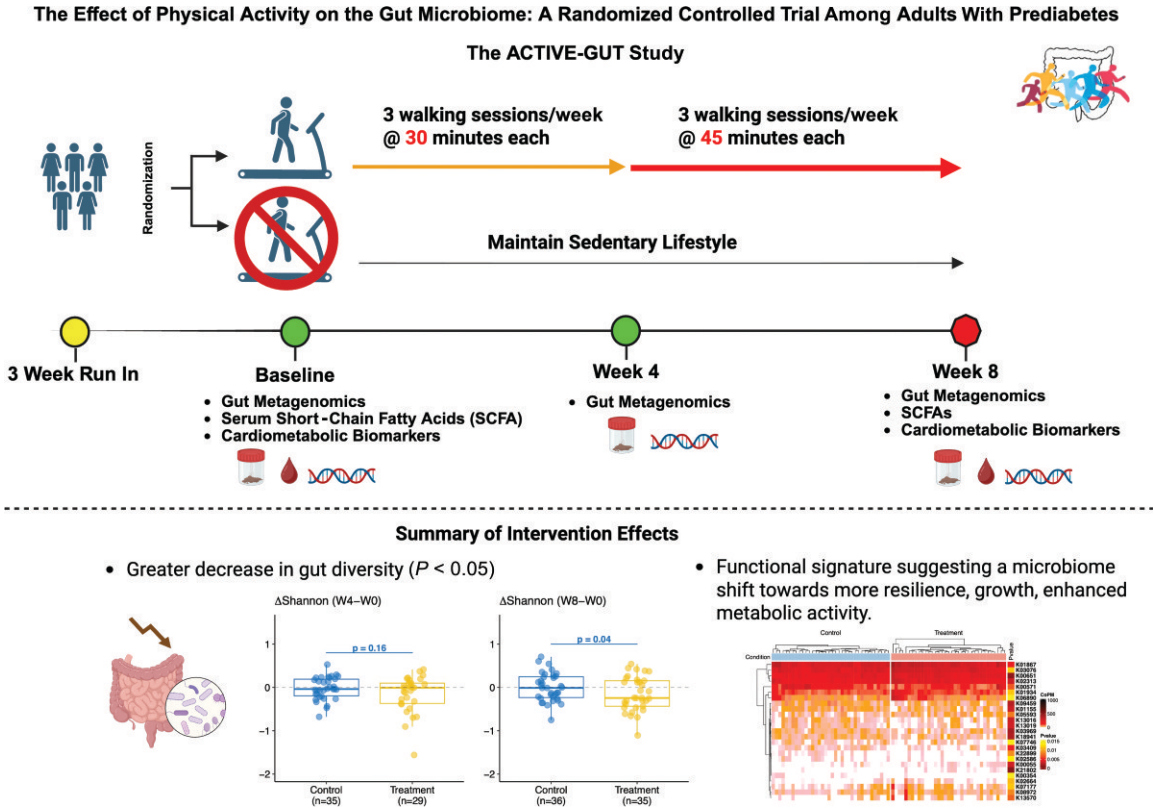

ARTICLE HIGHLIGHTS

- **Why did we undertake this study?**
  The mechanisms linking physical activity to improved health are not fully understood. The gut microbiome is one hypothesized intermediate linking physical activity to improved health.

- **What is the specific question we wanted to answer?**
  Does moderate physical activity alter the gut microbiome and circulating short-chain fatty acids among sedentary adults with prediabetes and overweight or obesity?

- **What did we find?**
  Individuals randomized to 8 weeks of moderate-intensity walking realized a greater decrease in gut microbiome diversity compared with inactive control individuals. At the functional levels, these changes were suggestive of increased microbiome resilience, growth, and enhanced metabolic activity.

- **What are the implications of our findings?**
  These findings shed new insights into the mechanisms linking physical activity to improved metabolic health.

ORIGINAL ARTICLE

The Effect of Physical Activity on the Gut Microbiome in Prediabetes: Results From a Randomized Controlled Trial

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OBJECTIVE | To test the effect of physical activity on the gut microbiome and circulating short-chain fatty acids (SCFAs) among sedentary adults with prediabetes and overweight or obesity.

RESEARCH DESIGN AND METHODS | In a pilot and feasibility trial, we randomized 77 adults with prediabetes and a sedentary lifestyle into one of two groups: *1*) intervention: invited to engage in home-based moderate-intensity walking 3×/week for 30 min/session in weeks 1–4 and for 45 min/session during weeks 5–8 of the 8-week intervention; or 2) control: maintained habitual physical activity levels. We performed metagenomic sequencing from stool collected at baseline, week 4, and week 8, with SCFAs measured from serum collected at baseline and week 8. Taxonomic and functional profiling was performed on the metagenomic reads; α-diversity metrics were subsequently derived. Linear regression assessed the difference in change between the intervention and control groups for α-diversity and SCFA levels.

RESULTS | We screened 1,533 participants for eligibility and consented 132. Of these, 87 entered the run-in phase, and 77 were randomized. Participants were mean (SD) 51.4 (8.9) years old, 87.7% female, and 74% non-Hispanic White. Mean fasting glucose was 103.3 (13.2) mg/dL, while mean BMI was 34.4 (5.7) kg/m^2. Compared with the control group, the intervention group experienced decreased α-diversity as characterized by Shannon, Richness, and Faith diversity indices by intervention week 8 ($P < 0.05$). Changes in SCFA levels were not statistically significant different in intervention versus control.

CONCLUSIONS | Randomization to a walking intervention resulted in modest gut microbiome changes among adults with overweight or obesity and prediabetes.

Prediabetes affects nearly half of U.S. adults, totaling >115 million individuals (1). Up to 70% of individuals with prediabetes develop type 2 diabetes (T2D), and both prediabetes and T2D heighten cardiovascular disease (CVD) risk (1). Physical activity (PA) is a key modifiable determinant of good health. The Diabetes Prevention Program (DPP) found that among those with prediabetes, a behavioral intervention emphasizing PA and diet was at least as successful as metformin in preventing T2D, partially due to weight loss (2). Yet, many individuals do not fully benefit from PA, and mechanistic pathways linking PA and cardiometabolic improvements are not fully understood, limiting the development of precision-oriented PA programs and/or adjunctive therapies to augment the benefits of PA (3).

The gut microbiome (4) has been posited as a mechanistic intermediate linking health behaviors, such as PA, to cardiometabolic disease development (5). The gut microbiome may be important for immunological (6,7), metabolic (4,8), and inflammatory (5) processes, some of which might partially explain how health behaviors influence cardiometabolic

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disease risk. These potential effects are possibly mediated through the anti-inflammatory effects of short-chain fatty acids (SCFAs) produced by gut microbiome metabolism of the host diet (7). However, limited evidence exists characterizing the effect of PA on the human gut microbiome. To date, most studies of the impact of PA interventions on the gut microbiome have used animal models, with results supporting regular chronic PA (i.e., exercise) as a modulator of the gut microbiota. Human trials (9–11) also generally report gut microbiota compositional changes following PA, but existing studies suffer from lack of randomization and control groups and have often consisted of metabolically healthy individuals.

To address existing knowledge gaps, we conducted a pilot randomized controlled parallel trial to test the influence of walking exercise on the gut microbiome and circulating SCFAs among sedentary individuals with prediabetes and overweight or obesity. We hypothesized that randomization to the intervention group's 8-week walking program would alter the gut microbiome composition relative to the control group.

Research Design and Methods

We conducted a randomized controlled parallel pilot and feasibility trial to test the influence of regular walking exercise on the gut microbiome and circulating SCFA. Inclusion criteria were *1*) 30–64 years of age; *2*) BMI ≥25 kg/m^2; *3*) prediabetes, defined as a fasting glucose of 100–125 mg/dL, a 2-h oral glucose tolerance test of 140–199 mg/dL, or an HbA_{1c} of 5.7–6.4%; *4*) currently engaged in <100 min/week of physical activity as assessed via the Modifiable Activity Questionnaire (12); *5*) stable weight (<10% change) over the last 6 months; *6*) willing to maintain current dietary and exercise habits aside from any changes made per the study exercise protocol; and *7*) willing to be randomized to one of the two study groups. Exclusion criteria were *1*) any exercise contraindications identified via the Physical Activity Readiness Questionnaire (13); *2*) physical or mental disabilities that would limit adherence to the intervention or gastrointestinal conditions that would interfere with the primary outcome (e.g., Crohn disease); *3*) antibiotic use in the past 45 days; *4*) history of bariatric surgery or other similar medical interventions that would interfere with the primary outcome; or *5*) currently pregnant, planning to becoming pregnant, or breastfeeding. Participant flow is summarized in the Consolidated Standards of Reporting Trials (CONSORT) diagram (Supplementary Fig. 1). All participants provided written informed consent, and the study was approved by the University of Minnesota and Mayo Clinic Institutional Review Boards. The trial protocol is registered at clinicaltrials.gov NCT04931836.

Home-Based Walking Intervention

Participants were randomized into an intervention or control group based on a randomization scheme developed and delivered by a research team member (N.R.M.). The intervention group engaged in an 8-week home-based walking intervention. Briefly, during the 8-week intervention, intervention group participants were asked to engage in home-based moderate-intensity walking 3×/week for 30 min/session in weeks 1–4 and for 45 min/session during weeks 5–8. We also asked intervention group participants to engage in at least 6,000 steps/day on days they chose to walk for the trial. They monitored their activity with a Fitbit Inspire 2. When factoring in participants' activities of daily living, this intervention was meant to assist participants in achieving the 150 min/week of moderate-intensity aerobic activity recommended by the Physical Activity Guidelines for Americans. Notably, this exercise dose (i.e., intensity × session duration × weekly frequency × intervention length) is equivalent to or greater than that used in most existing human studies of exercise and the gut microbiome as well as the exercise dosage observed necessary to yield meaningful cardiometabolic changes (14). Control group participants were asked to maintain their usual PA during the 8-week intervention period and were provided a Fitbit Inspire 2 so that we could monitor overall levels of activity in this group. They were allowed to keep this device following study completion. All Fitbit data were synced with Fitabase, a cloud-based platform for obtaining Fitbit/Garmin data and monitoring compliance; the latter allowing us to prompt adherence to the walking intervention among intervention group participants.

Microbiome Measurement and Data Processing

Participants collected stool samples at home with an OMNIgene-GUT OM-200 stool collection kit (DNA Genotek, Ottawa, Ontario, Canada). Single samples were collected within 14 days prior to randomization and at weeks 4 and 8 following randomization. Participants' food intake was logged for 3 days prior to the first stool sample collection. They were then asked to consume these same foods prior to providing stool samples at weeks 4 and 8. Participants returned samples to the laboratory within 30 days for storage at −80°C. An Epmotion robot extracted DNA from stool aliquots using the 96-well plate Mo Bio Power soil kit. DNA was eluted into 50-μL aliquots. All extractions contained negative and positive controls representing defined bacterial communities (Zymo, Irvine, CA). DNA was quantified using Quant-iT and stored at −80°C. Metagenomic libraries were prepared at the University of Minnesota Genomics Center using the Nextera XT kit (Illumina, San Diego, CA), and barcoded. DNA libraries were denatured with NaOH and diluted to 8–12 pmol/L in Illumina's HT1 buffer, spiked with 1% PhiX and a HiSeq 1 × 100 cycle v3 ki Illumina HiSeq.

Shotgun metagenomics reads were processed by first running BBDuk to remove any remaining adapter sequences, low-quality read regions, phiX sequences, and low-complexity/low-entropy reads (15). BioBloomTools was used to filter out host reads, with hg38 used as a reference (16). Taxonomic profiling of the remaining shotgun

metagenomics reads was performed using Sourmash (version 4.2.4) using the "sourmash gather" and "sourmash taxonomy" commands with Genome Taxonomy Database (GTDB202) used as a reference database to estimate the relative abundances of each taxon in each sample (17). Functional profiling of the shotgun metagenomics reads was performed by using HMP Unified Metabolic Analysis Network (HUMAnN3; v3.6) with the Struo2 release of the GTDB202 database formatted for HUMAnN3 used as a reference database (18,19). Gene families identified by HUMAnN3 were mapped to MetaCyc pathways and normalized to copies per million using the utility scripts packaged with HUMAnN3.

SCFA Assessment

Metabolon (Durham, NC) measured SCFA from serum collected at baseline and week 8 using a previously published pipeline (20). Briefly, serum samples were spiked with a solution of eight stable labeled internal standards and subjected to protein precipitation. After centrifugation, an aliquot of the supernatant was derivatized. The reaction mixture was analyzed by liquid chromatography–tandem mass spectrometry on an Agilent 1290/AB Sciex 5500 system (AB Sciex LLC, Marlborough, MA). Peak areas of the respective analyte product ions are measured against the peak area of the corresponding internal standard product ions. A weighted least-squares regression analysis performed quantitation against fortified calibration standards prepared immediately prior to each run.

Risk Factor Assessment

At baseline, trained researchers collected covariates by administering validated questionnaires about participant self-reported age, sex (male/female), race and ethnicity (Black non-Hispanic, White non-Hispanic, Hispanic, other), and educational attainment (less than high school, high school or equivalent, more than high school). Trained researchers also completed anthropometric measurements, including height and weight (from which BMI was calculated), estimated percentage of body fat, heart rate, and blood pressure using an Omron automated blood pressure monitor (Kyoto, Japan). Phlebotomists collected blood at baseline and week 8 following an overnight fast. Colleagues at the University of Minnesota's Advanced Research and Diagnostics Laboratory assayed plasma glucose, insulin, and serum lipids at baseline and week 8 using Roche assays on a cobas automated analyzer according to the manufacturer's instructions.

Statistical Analysis

All analyses were conducted in SAS 9.4 or R 4.2.2. Phyloseq was used to calculate α-diversity indices, rarefied at a depth of 500,000 reads, and analyzed using linear models. Taxonomic differential abundance analysis was performed using ZicoSeq (21). Taxa with a prevalence <10% or with a maximum proportion of <0.2% were excluded from testing to reduce the number of tests. Functional differential abundance analysis was performed using MaAsLin2. Minimum feature relative abundance was set to 2×10^{-4}; all other parameters were set to default values. False discovery rate (FDR) control (B–H procedure 48) was used to correct for multiple testing, and FDR-adjusted P values (i.e., q values) <0.1 were considered significant. Generalized linear regression models were used to regress biomarker changes on group allocation status. Nonnormal variables were natural log transformed prior to analysis. With a 0.4-unit SD in the Shannon index and sample size of 72, we had 80% to detect a difference in change of 0.29 Shannon units.

Data and Resource Availability

The data sets generated during and/or analyzed in the current study are available from the corresponding authors upon reasonable request.

Results

Among 1,533 participants screened for eligibility, 132 consented, and 87 were enrolled and started the run-in phase, with 77 being randomized (Supplementary Fig. 1). Randomized individuals were mean (SD) 51.4 (8.9) years old, 87.7% female, and 74% non-Hispanic White. Mean fasting glucose was 103.3 (13.2) mg/dL, and mean BMI was 34.4 (5.7) kg/m^2 (Table 1). Participant characteristics were well balanced between the intervention and control groups. The mean (SD) α-diversity metrics of the Shannon index and Faith index were 3.60 (0.47) and 51.1 (13.7), respectively, overall. The taxonomic profile of participants by intervention group is presented in Fig. 1.

Effect of Intervention on Daily Step Count and Cardiometabolic Risk Biomarkers

We present mean steps per day at baseline, week 4, and week 8 in Table 2. Baseline mean (SD) steps per day among the intervention and control groups were 5,856 (2,582) and 5,813 (2,206) ($P > 0.05$), respectively. From baseline to week 8, mean steps per increased by 480 (95% CI −223, 1,185) steps among the intervention group compared with the control group. Baseline levels of HbA_{1c}, blood pressure, fasting cholesterol, triglycerides, glucose, and insulin are presented in Table 1. We observed no difference in changes for any of the cardiometabolic risk biomarkers during the intervention period (Table 2).

Effect of Intervention on SCFA

SCFA levels are presented by time point along with mean differences between groups in Table 2. No statistically significant differences in SCFA profiles were observed between

TABLE 1 Baseline characteristics of ACTIVE-GUT study participants

	All (*N* = 77)	Intervention group (*n* = 39)	Control group (*n* = 38)
Age (years)	51.43 (8.95)	49.85 (9.25)	53.05 (8.45)
Male sex, %	14.29	12.82	15.79
Race and ethnicity, %			
Hispanic	7.79	5.13	10.53
Non-Hispanic Black	6.49	5.13	7.89
Non-Hispanic White	74.03	79.49	68.42
Other	11.69	10.25	13.16
Weight (kg)	98.07 (20.46)	99.01 (21.48)	97.11 (19.62)
BMI (kg/m^2)	34.4 (5.72)	34.66 (6.09)	34.14 (5.4)
Body fat (%)	43.75 (6.92)	44 (7.17)	43.49 (6.74)
Blood pressure			
Systolic (mmHg)	123.31 (13.22)	124.58 (13.11)	122 (13.39)
Diastolic (mmHg)	83.37 (9.45)	85.53 (8.90)	81.16 (9.60)
Pulse (beats/min)	74.17 (8.88)	74.47 (8.33)	73.86 (9.51)
Step count (steps/day)	5,913.45 (2,386.9)	5,818.74 (2,476.58)	6,013.27 (2,318.45)
HbA_{1c} (%)	5.90 (0.20)	5.89 (0.20)	5.92 (0.20)
Fasting glucose (mg/dL)	103.32 (13.32)	101.05 (11.76)	105.66 (14.53)
Fasting Insulin (μU/mL)	125.92 (83.69)	126.28 (80.78)	125.55 (87.66)
LDL cholesterol (mg/dL)	125.7 (39.39)	118.1 (32.44)	133.5 (44.54)
HDL cholesterol (mg/dL)	52.42 (13.39)	51.79 (13.94)	53.05 (12.96)
Triglycerides (mg/dL)	145.58 (70.91)	141.97 (66.03)	149.29 (76.31)
C-reactive protein (mg/L)	5.74 (6.33)	6.54 (7.79)	4.91 (4.3)
Acetic acid (ng/mL)	2,297.56 (1,181.96)	2,352.79 (1,217.36)	2,240.87 (1,158.05)
Propionic acid (ng/mL)	52.92 (26.71)	53.36 (25)	52.47 (28.7)
Butyric acid (ng/mL)	34.82 (43.19)	29.45 (12.17)	40.32 (60.15)
2-Methyl-butyric acid (ng/mL)	18.69 (7.58)	18.34 (7.72)	19.04 (7.52)
Isobutyric acid (ng/mL)	27.62 (20.05)	26.04 (14.18)	29.24 (24.78)
Valeric acid (ng/mL)	7.59 (4.4)	7.37 (3.24)	7.82 (5.37)
Isovaleric acid (ng/mL)	35.53 (23.4)	35.53 (22.14)	35.52 (24.92)
Hexanoic acid (ng/mL)	39.72 (44.98)	36 (13.83)	43.54 (62.68)
Shannon index	3.60 (0.47)	3.57 (0.48)	3.63 (0.46)
Faith index	51.07 (13.7)	50.61 (12.93)	51.50 (14.44)

Data are presented as mean (SD) unless otherwise indicated.

the intervention and control groups. Observations were consistent in analyses adjusting for baseline SCFA levels.

Effect of Intervention on Gut Microbiome

The intervention resulted in decreased α-diversity, as characterized by Shannon, Richness, and Faith, by week 8 of the intervention (Fig. 2, Supplementary Fig. 2, and Supplementary Tables 2 and 3). Additionally, inverse Simpson was decreased by week 4 in the intervention group ($P < 0.05$), but this observation was not significant by week 8. The top signals from exploratory analyses at the levels of taxa, functional pathways, and/or Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology (KO) groups are shown in Fig. 2 and Supplementary Figs. 3 and 4. The relative abundance of butyrate producers *Coprococcus catus*, *Agathobaculum sp003481705*, and *Butyricimonas faecihominis* were differentially abundant following exercise, although only *B. faecihominis* was increased in the intervention group (Supplementary Fig. 3 and Supplementary Table 3). Surprisingly, relative abundance of *Roseburia hominis* was marginally statistically significantly reduced in the intervention group ($P = 0.06$). After adjustment for multiple comparisons, there were no statistically significant differences at the levels of taxa or functional pathways. Phylum level taxonomic composition did not change across study time points (Fig. 1). Figure 2 shows results for analyses of KOs, where there were also no results

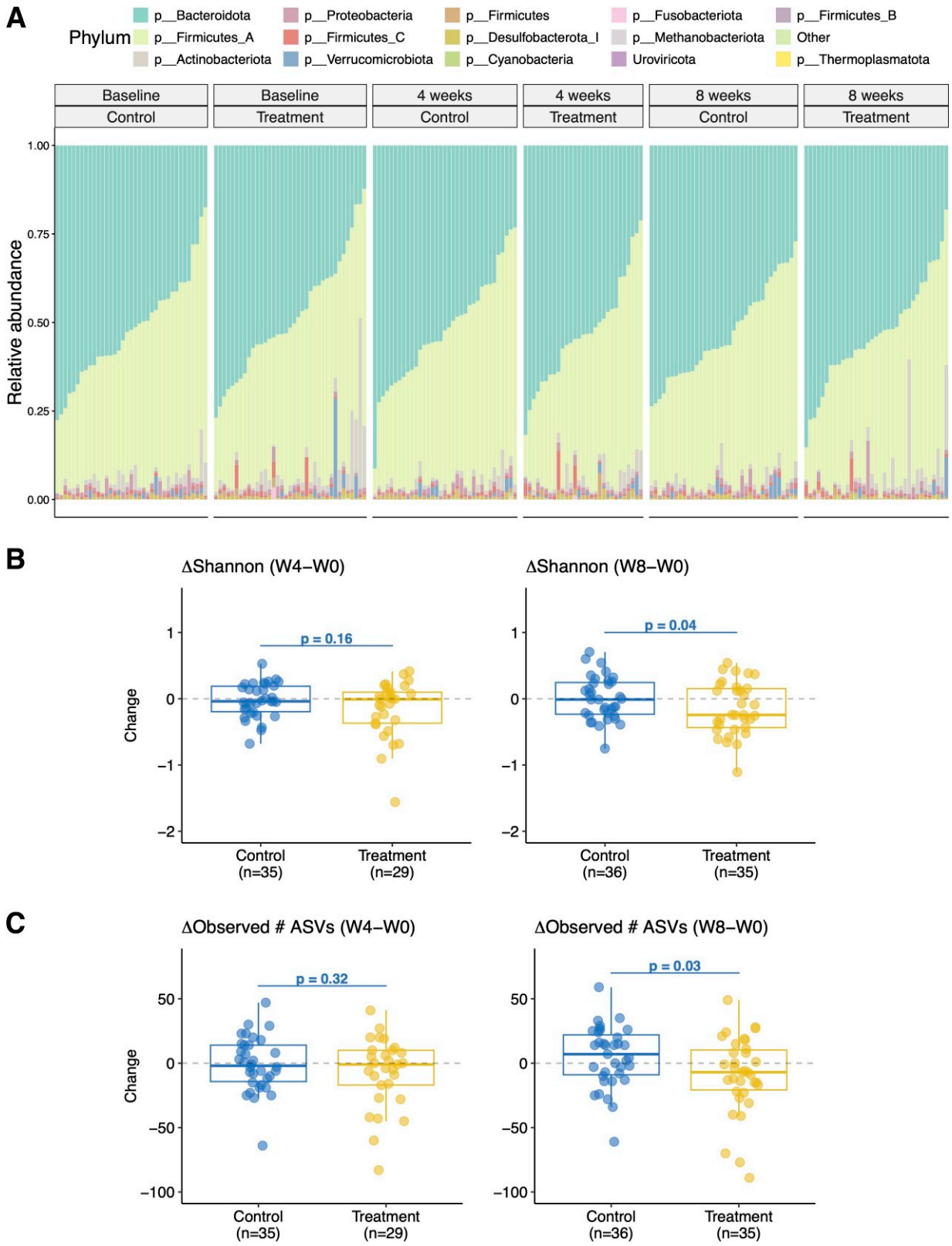

FIGURE 1 Phylum-level taxonomic profiles (*A*), change in Shannon index (*B*), and change in observed number of taxa (*C*) at baseline, week 4, and week 8 among 77 participants in the ACTIVE-GUT trial. Analyses for change in Shannon or observed (*B* and *C*) are adjusted for baseline levels of α-diversity.

TABLE 2 Longitudinal change in step count, serum SCFA, and cardiometabolic risk biomarkers by intervention group

	Intervention group (n = 33)			Control group (n = 37)			
Variable	Baseline	Week 4	Week 8	Baseline	Week 4	Week 8	Difference in change (95% CI)*
Steps/day	5,856 (2,582)	6,511 (2,708)	6,349 (2,870)	5,813 (2,206)	5,930 (2,320)	5,831 (2,211)	480.8 (−223.8, 1,185.4)
SCFA							
Acetic acid (ng/mL)	2,408 (1,311)	NA	2,326 (957)	2,260 (1,168)	NA	2,021 (745)	218.05 (−136.33, 572.44)
Propionic acid (ng/mL)	54.30 (24.11)	NA	57.19 (24.49)	52.92 (28.96)	NA	54.04 (24.86)	1.35 (−8.21, 10.91)
Butyric acid (ng/mL)	28.94 (11.94)	NA	30.95 (18.12)	41.05 (60.81)	NA	41.74 (61.89)	0.91 (−7.50, 9.31)
2-Methylbutyric acid (ng/mL)	18.74 (8.18)	NA	18.82 (9.78)	19.12 (7.61)	NA	18.39 (5.56)	−0.18 (−2.02, 1.67)
Isobutyric acid (ng/mL)	26.56 (15.04)	NA	26.71 (16.51)	29.32 (25.11)	NA	28.50 (11.80)	−1.80 (−4.55, 0.95)
Valeric acid (ng/mL)	7.41 (3.32)	NA	7.01 (3.69)	8.11 (5.37)	NA	7.07 (4.20)	−0.14 (−1.82, 1.54)
Isovaleric acid (ng/mL)	35.55 (21.42)	NA	37.20 (30.12)	33.95 (23.28)	NA	32.68 (17.80)	2.69 (−6.54, 11.91)
Hexanoic acid (ng/mL)	35.88 (14.69)	NA	39.21 (31.47)	44.03 (63.48)	NA	40.35 (49.28)	0.58 (−3.98, 5.15)
Cardiometabolic risk biomarkers							
Weight (kg)	96.32 (18.05) [34]	97.94 (17.86) [30]	96.65 (17.94) [34]	96.49 (19.52) [36]	95.35 (18.45) [35]	96.43 (19.60) [36]	0.39 (−0.31, 1.09)
BMI (kg/m^2)	34.09 (5.19) [33]	34.70 (5.16) [29]	34.22 (5.16) [33]	34.12 (5.48) [36]	33.81 (5.16) [35]	34.09 (5.48) [36]	0.15 (−0.10, 0.40)
Body fat (%)	43.38 (7.19) [34]	43.40 (7.51) [30]	43.21 (7.51) [34]	43.87 (6.42) [36]	43.44 (7.19) [35]	43.86 (6.64) [36]	−0.18 (−1.32, 0.97)
Blood pressure							
Systolic (mmHg)	123.32 (12.93) [34]	123.00 (12.24) [30]	121.21 (14.43) [34]	122.19 (13.52) [36]	122.09 (14.12) [35]	119.58 (14.65) [36]	0.79 (−4.39, 5.97)
Diastolic (mmHg)	84.53 (8.78) [34]	84.07 (8.52) [30]	84.71 (11.49) [34]	81.14 (9.73) [36]	81.09 (12.28) [35]	79.58 (10.09) [36]	2.29 (−1.38, 5.95)
Pulse (bpm)	74.00 (8.07) [34]	74.10 (8.01) [30]	74.26 (8.57) [34]	73.58 (9.48) [36]	71.57 (8.48) [35]	72.03 (9.53) [36]	1.96 (−1.35, 5.27)
Fasting glucose (mg/dL)	101.73 (11.97) [33]	NA	103.52 (13.61) [33]	105.19 (13.20) [36]	NA	106.42 (13.00) [36]	0.007 (−3.92, 3.94)
Fasting insulin (μU/mL)	118.09 (73.62) [32]	NA	134.25 (81.58) [32]	114.72 (60.57) [36]	NA	133.22 (85.57) [36]	−2.34 (−26.93, 22.25)
LDL cholesterol (mg/dL)	118.18 (34.50) [33]	NA	120.48 (34.25) [33]	133.32 (45.14) [37]	NA	124.49 (44.86) [37]	9.57 (1.06, 18.08)
HDL cholesterol (mg/dL)	51.18 (14.81) [33]	NA	52.33 (14.87) [33]	53.38 (12.98) [37]	NA	52.68 (11.60) [37]	1.65 (−0.37, 3.66)
Triglycerides (mg/dL)	145.82 (70.53) [33]	NA	147.76 (77.77) [33]	144.84 (72.19) [37]	NA	142.38 (62.08) [37]	4.60 (−14.85, 24.04)
C-reactive protein (mg/L)	4.83 (3.85) [31]	NA	4.54 (3.37) [31]	4.96 (4.35) [37]	NA	4.58 (3.62) [37]	0.049 (−0.93, 1.03)

Data are mean (SD) unless otherwise noted, and data in brackets are n. Data are missing for step counts for n = 2 in the control group. Data are missing for valeric acid for n = 1 among the intervention group and n = 2 among the control group. Sample sizes for cardiometabolic biomarkers by intervention vs. control and time point are presented in the respective cells. NA, not available. *Difference in change defined as week 8 value minus baseline and values are adjusted for baseline level of the outcome.

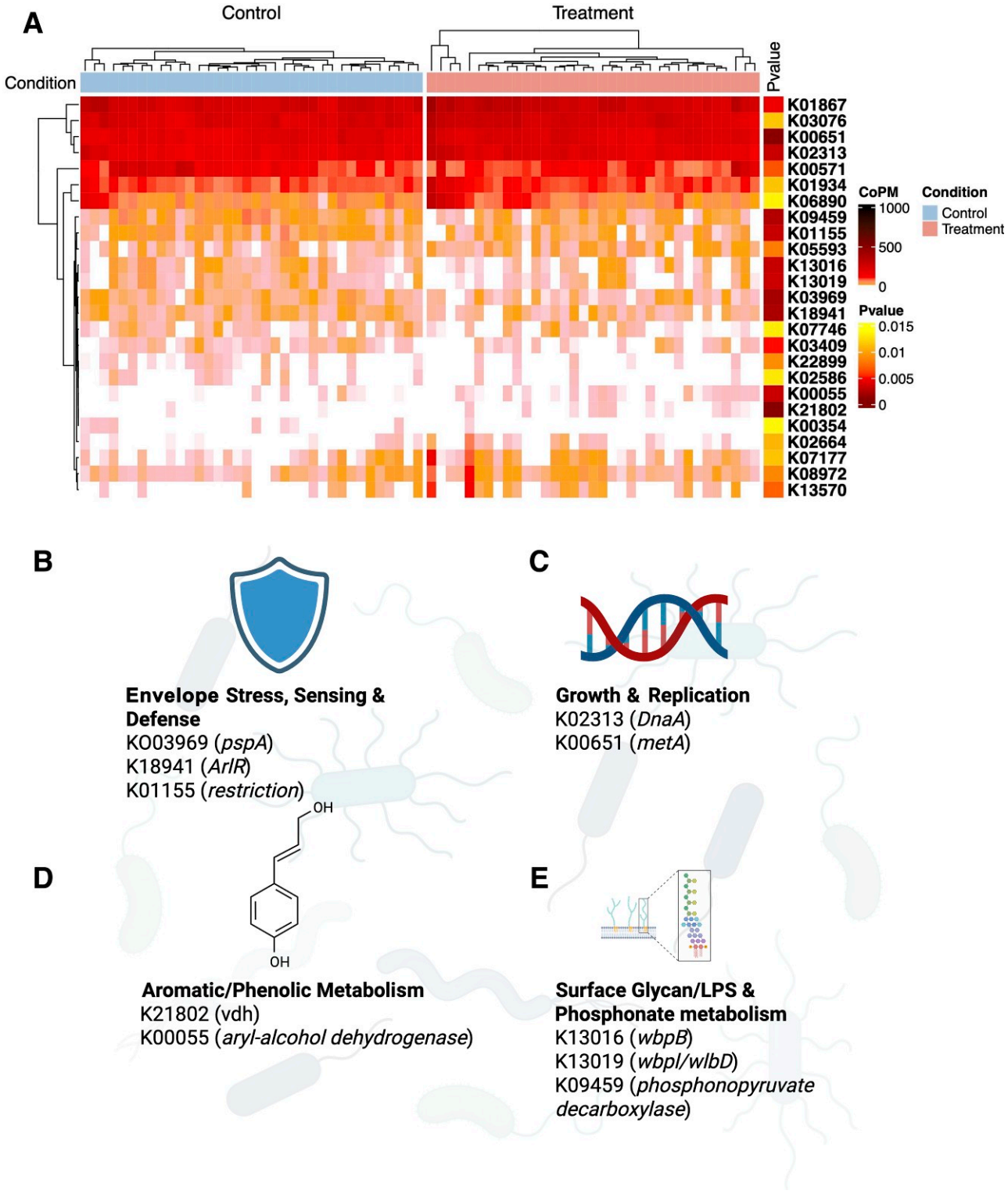

FIGURE 2 Top differentially abundant KO pathways at week 8 by intervention status. *A*: Heat map ranked by raw *P* value with four themes highlighted: envelope stress, sensing, and defense (*B*); growth and replication (*C*); aromatic/phenolic metabolism (*D*); and surface glycan/lipopolysaccharide (LPS) and phosphonate metabolism (*E*). Collectively, these themes suggest a microbiome that is more resilient, replicative, and surface-retooled while enhancing polyphenol catabolism.

with an FDR <5%; however, the top KO (K00651) had an FDR of 16% and among the top 10 KOs, all had an FDR <30% (suggesting only three false positives among the top 10). All had raw *P* values < 0.005. The KOs altered involved molecular functions related to envelope stress, sensing, and defense (K003969 and K01155); growth and replication

capacity (K02313 and K00651); cell-surface glycan/lipopolysaccharide and phosphonate metabolism (K13016, K13019, and 09459); and aromatic/phenolic metabolism (K21802 and K00055). These are all themes consistent with a microbiome with enhanced metabolic activity and host-relevant metabolic signaling.

No differences in β-diversity metrics were observed between intervention and control groups by week 8 (Supplementary Fig. 5).

Conclusions

We conducted a randomized controlled parallel pilot and feasibility trial to test the influence of moderate-intensity walking exercise on the gut microbiome and circulating SCFAs among individuals with prediabetes. We observed greater decreases in gut microbiome α-diversity measures in the intervention versus control group. While we were underpowered to detect an interventional effect on individual taxa, functional pathways, KOs, or SCFA changes due to multiple comparisons, these data can inform the design of future trials powered for these outcomes.

To our knowledge, this is the first randomized controlled trial to assess the effect of moderate physical activity on the gut microbiome among individuals with prediabetes and overweight or obesity. Our observation that gut diversity was reduced is counter to our expectations based on prior studies reporting that gut diversity is enriched among individuals who are more PA and/or who have better cardiometabolic health (22). Results from prior animal studies have typically observed exercise-related increases in gut α-diversity (23,24). Prior studies in adults with overweight or obesity (9–11) also found increased α-diversity and altered β-diversity after exercise; similar findings emerged in another study of healthy older adults (25). Despite our seemingly counterintuitive findings in which α-diversity decreased in the intervention arm, there is also a body of evidence to suggest that short-term decreases in gut diversity following the initiation of lifestyle, particularly dietary, interventions could be expected and beneficial. Numerous prior studies of dietary fiber supplementation report decreased α-diversity following fiber supplementation as nicely reviewed by Cantu-Jungles and Hamaker (22). Moreover, results from clinical trials report that, even in the presence of decreased gut α-diversity, improvements in several cardiometabolic biomarkers, including measures of adiposity, glucose control, and appetite control, emerged (22). Animal models of antibiotic-induced microbial depletion have similarly shown short-term depletion in α-diversity is concurrent with improvements in cardiometabolic biomarkers (26). Nevertheless, the causal nature of the relationship between α-diversity and biomarkers remains unresolved, as the pleiotropic effects of dietary fiber, antibiotics, or exercise could affect biomarkers through pathways unrelated to the interventional effects on the gut microbiome. Future research will be necessary to better identify whether short-term decreases in gut α-diversity are transient and/or causally linked to improved cardiometabolic health.

If the relationships are causal, the observed magnitude of effect on α-diversity change is potentially biologically meaningful. In a prior study of heart failure patients, the cross-sectional difference in Shannon diversity between patients with versus without recent antibiotic exposure (among the most powerful interventions known to modify microbial diversity) was 0.56 units, which equated to a 10% difference in that sample (27). Presently, we report an ∼5% difference in Shannon index change following an unsupervised home-based walking intervention among the intervention group versus the control group.

Biological Plausibility

Several mechanisms potentially explain how exercise might favorably alter the gut microbiome. Once altered, the gut microbiome is believed to have an important role in immunological (7) and metabolic functioning (4,8), as well as inflammatory processes (7,28), all of which are important for cardiometabolic health. Exercise can alter gene expression of gut-associated lymphoid tissue (GALT), downregulating inflammation and oxidative stress (29). Given the close proximity of GALT to gut microbial communities, these effects influence host-microbial homeostasis (30). Exercise can also affect the gut mucus layer, providing substrate to beneficial *muciniphilic* taxa (e.g., *Akkermansia muciniphila*) and also by aiding with improved gut barrier integrity, possibly counteracting translocation of proinflammatory microbial byproducts (31). Exercise could also enrich SCFA-producing taxa (24,32,33), which has been demonstrated in animal studies. Interestingly, a prior study in humans suggested that exercise may increase the abundance of *Veillonella atypica* (34), which likely occurs because *Veillonella atypica* uses excess lactate produced during exercise as an energy source and metabolizes lactate to produce the SCFAs acetate and propionate (34). Propionate is known to benefit cardiac function and improve maximum oxygen consumption and blood pressure in mice (35,36) and to increase resting energy expenditure in fasting humans (37). In our current study, we observed greater propionate elevations in the intervention versus control group, although this analysis was underpowered and not statistically significant. Conceptually, it is possible that an exercise-induced positive feedback loop could develop in which exercise-induced host lactate production leads to the enrichment of lactate-reducing taxa and SCFA production, resulting in improved exercise tolerance. Our current results show that the propionate producer *Rosburia hominis* (which also enhances innate immune function) was a top taxon altered in the intervention group (although not significantly and it was unexpectedly depleted in the intervention group). Similarly, the butyrate producers *Coprococcus catus*, *Agathobaculum sp003481705*, and *Butyricimonas faecihominis* were differentially abundant in

the intervention versus control group; however, these taxa were not differential after adjustment for multiple comparisons. Larger studies with more intense exercise and more frequent assessments of the microbiome and SCFA production are needed to explore changes at the taxonomic level that could support or refute this hypothesis.

Our observations relevant to microbial molecular function (KO results), although underpowered, yielded results with lower risk of false discovery that are consistent with the anticipated impact of increased host physical activity (i.e., walking) on the gut microbiome. As such, they are worth brief discussion as they provide insights into future investigation. First, the results suggest a shift toward a more metabolically active gut microbiome with a metagenome enriched for microbial growth, replication, and biosynthesis (KOs K02313 and K00651). Second, the signal suggesting less aromatic/phenolic metabolism (K21802 and K00055) and production of phenolic metabolites from dietary polyphenols is noteworthy. Phenolic acids can upregulate tight-junction proteins and improve insulin signaling, while reducing epithelial oxidative stress and nuclear factor-κB signaling (38–40). While these functions are generally beneficial, a shift away from host-protective functions could simply reflect an ecology-dependent shift in metabolism if walking increased upper-gut absorption and allowed fewer polyphenol-rich substrates to reach colonic microbes. Alternatively, phenolic metabolism responsibility may have shifted to other taxa or to host-dependent pathways. Finally, depletion of microbial envelope stress, sensing, and defense functions potentially reflects lower luminal stress (e.g., reduced mucosal inflammation) and/or community replacement forcing a compositional shift away from pathogens/pathobionts. Together, the top 10 KOs are consistent with a microbiome adapting to a walking exercise altered gut milieu; particularly, a milieu exposed to less luminal stress, with greater replication and biosynthetic capacity. However, the signal is general and should be interpreted alongside other functional and taxonomic changes in animal models and larger human studies to clarify whether it represents beneficial bacterial growth or a microbial shift toward specific metabolic pathways.

Limitations and Strengths

Our study has some important limitations and strengths. We were limited by a relatively modest interventional effect on daily step count, which was hampered by the coronavirus disease pandemic occurring during the study, and we lacked information on the intensity of the increased step count. While the baseline participant average steps per day were typical for the average adult, there is ample room for increasing walking and achieving related health benefits in this sample (41). Future fully powered, larger trials are needed, with improved walking adherence. Our pilot study was small and underpowered for more detailed mechanistic investigations at the taxonomic and metagenomic level, and future studies with sufficient power for omics outcomes will be informative. Important strengths include our use of a randomized controlled parallel design to minimize confounding and a repeated-measures design that informs the time window necessary for exercise to produce a change in the gut microbiome.

Conclusion

In a randomized controlled trial, we have observed a modest decrease in gut microbial α-diversity among participants randomized to an 8-week walking intervention versus a control group. If confirmed in larger studies, our results suggest that modest increases in activity could be part of broader interventions targeting gut health. Future research should explore whether baseline gut composition possibly synergizes with exercise to produce health effects and/or whether exercise-induced changes in the gut microbiome mechanistically translates into improved cardiometabolic health during longer follow-up periods. Understanding these dynamics could help inform the heterogeneity of exercise effects on cardiometabolic and other health outcomes and potentially holds promise for the development of precision-oriented exercise interventions.

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During the course of preparing this work, the authors used ChatGPT for the purpose of fixing grammar, clarity, and active voice. After using this tool/service, the authors formally reviewed the content for its accuracy and edited it as necessary. The authors take full responsibility for all the content of this publication.

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DUALITY OF INTEREST

No potential conflicts of interest relevant to this article were reported.

AUTHOR CONTRIBUTIONS

R.T.D. drafted the original manuscript. R.T.D., Z.C.P., A.J., and M.A.P. contributed to concept and study design. R.T.D., N.R.M., and S.J. analyzed the data. F.R.R.A., P.F.R.H., S.S., D.J.M., and S.A.R. contributed to data collection. All authors made appropriate contributions toward authorship and approved the content of the manuscript. All authors contributed to interpretation of results and to reviewing the final version of the manuscript. R.T.D. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

PRIOR PRESENTATION

Parts of this study have been submitted in abstract form to the Scientific Sessions 2026 of the American Heart Association, Chicago, IL, 6–9 November 2026.

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